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THE BACTERIOLOGY OF CHRONIC EMPYEMA

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The development in the treatment of empyema from a purely surgical procedure to one involving the postoperative use of antiseptic solutions, has caused an increased interest in the bacteriology of that condition. Accurate bacteriologic information is recognized as of importance both from the standpoint of etiology and prognosis, and in the subsequent control of treatment.

The bacteriology of initial empyema, in civil practice, indicates that the pneumococcus is the organism most commonly involved as the causative agent, with the ordinary pus organisms next in importance, and finally the tubercle bacillus.[1] In the reports of various series of cases from army camps, however, a number of instances are recorded in which no case of pneumococcal origin has been observed. In general, the average incidence of pneumococcus empyema throughout the service will probably not be more than 20% of reported cases.

The unusual occurrence of streptococcus pneumonia during the past two winters, especially that of 1917-1918, has undoubtedly accounted for the fact that the predominating organism in empyema, as occurring in military practice, has been the streptococcus. In the majority of cases, the streptococcus has been of the hemolytic variety.[2]

In addition to the two main bacteriologic types of the infection, pneumococcus and streptococcus empyema, scattering reports are found in the literature in which the etiology is ascribed to a variety of organisms; to B. paratyphosus A,[3] B. paratyphosus B,[4] the meningococcus,[5] the gonococcus,[6] the staphylococcus,[7] and to actinomyces.[8] In addition, empyema due to B. influenzae of Pfeiffer, and to psorosperms are on record. Thus it must be recog-

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[1] Osler: The Principles and Practice of Medicine, 1918.

[2] Dunham and others: Jour. Am. Med. Assn., 1918, 71, p. 366; Hamburger and Mayers: ibid., 70, p. 915; Miller and Lusk: ibid., p. 702; Beals, Zimmerman and Marlow: J. Infect. Dis., 1918, 23, p. 475.

[3] Weeks, C. C.: Lancet, 1916, 2, p. 433.

[4] Roussel, Miramond de la Roquette and Fourcade: Bull. med. de l'Algérie, 1914, 25, p. 349.

[5] Rubenstone, A. I.: New York Med. Jour., 1914, 100, p. 975.

[6] Woodbury: Surg., Gynec. and Obst.: 1918, 27, p. 601.

[7] Harrigan, A. H.: Med. Rec., 1915, 88, p. 629.

[8] Shurly, B. R.: Tr. Climat. and Clin. Assn., 1914, 30, p. 196.

nized that while the common infecting agent in civil empyemas seems to be the pneumococcus, and the hemolytic streptococcus in the empyemas following the interstitial bronchopneumonia of the past two years, still empyema may on occasion be due to a wide variety of organisms.

BACTERIOLOGY OF CHRONIC EMPYEMA

Empyema must be recognized as a condition which may readily develop into the chronic state. Not only is the primary infection difficult to overcome, but secondary bacterial invasion is prone to ensue. With the opening of the chest cavity, the probability of secondary infection becomes almost assured. There is probably more gross infection in those cases of open pneumothorax which follow rib resection, than where thoracotomy with insertion of a relatively small tube is followed. But in either instance, it is evident that organisms may readily be aspirated into the pleural cavity. It is likewise evident that the flora thus introduced is likely to be diverse, and often representative of the saprophytic bacteria.

The presence of these secondary invaders has been recorded in the literature of empyema in several instances, without any direct reference to their nature except as "putrefactive bacteria."[9] Cole and MacCallum,[10] quote a particular case which at necropsy showed hemolytic streptococci in all cultures, as well as a gram-negative bacillus producing a putrefactive odor. They denote this putrefactive organism as being undoubtedly a terminal invader.

It seems clear that primary empyemic infection is in general due to one of two infecting micrococci, the pneumococcus and the streptococcus. And further, that following operation the bacterial flora is liable to undergo alteration, the pleura, already infected, being subjected to secondary invasion by organisms which confer on the discharge the fetid and putrescent odor so frequently observed in old empyema cases. A determination of the progressive bacterial picture or flora of these cases, as they develop chronicity, has formed the basis of this study.

CHRONIC EMPYEMA AT CAMP GORDON

The opportunity was afforded at this hospital of studying some 25 cases of empyema which had run a clinical course of from 2 months to as long as 17 months. Table 1 summarizes the histories of these cases. The date of admission, with the initial diagnosis, is given chronologically. A history of pneumonia, either primary or following measles or influenza (nasopharyngitis) is found to have preceded all cases with one exception. This case gave no history of an intercurrent pneumonia. The patient was admitted with measles, later developed a purulent otitis media, which was followed shortly

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[9] Graham and Bell: Am. Jour. Med. Sc., 1918, 156, p, 839; Bel, G. S.: New Orleans Med. & Surg. Jour., 1917, 70, p. 33. Goltman, M.: Memphis Med. Month., 1916, 37, p. 127.

[10] Jour. Am. Med. Assn., 1918, 70, p. 1146.

TABLE 1
PROGRESS RECORDS OF EMPYEMA CASES

Case No.	Date Admitted	Diagnosis on Admission	Pneumonia	Empyema	Status 4/1/19
1	12/23/17	Lobar pneumonia	12/23/17 Lobar, pneumo. 1	1/ 3/18 Hemolytic strep.	Free drainage
2	1/13/18	Measles, purulent otitis media 1/22/18	No history	1/23/18 Hemolytic strep.	Free drainage
3	4/ 5/18	Lobar pneumonia	4/ 5/18 Lobar, no culture	4/28/18 Hemolytic strep.	Discharged 2/24/19
4	7/16/18	Lobar pneumonia	7/16/18 Lobar, pneumo. I	7/27/18 Hemolytic strep.	Discharged 3/31/19
5	9/ 1/18	Measles	9/16/18 Lobar, streptoc.	9/24/18 Streptococcus	Closure 3/18/19
6	9/ 9/18	Measles	10/12/18 Lobar, hemolytic strep.	10/20/18 Hemolytic strep.	Free drainage
7	9/10/18	Measles	10/ 6/18 Lobar, hemolytic strep., pnc. IV	10/ 7/18 Hemolytic strep.	Discharged 3/15/19
8	9/18/18	Nasopharyngitis, acute	10/ 4/18 Lobar, hemolytic strep., pnc. IV	1/ 8/19 Hemolytic strep.	Discharged 3/23/19
9	9/23/18	Nasopharyngitis, acute bilateral	10/ 3/18 Bronchopneumonia, no culture	10/23/18 Hemolytic strep.	Free drainage
10	9/26/18	Nasopharyngitis	10/ 3/18 Lobar, hemolytic strep.	10/10/18 Hemolytic strep.	Free drainage
11	9/26/18	Nasopharyngitis, acute	10/ 9/18 Lobar, pneumo. II	10/27/18 Pneumococcus II	Free drainage
12	9/30/18	Lobar pneumonia	9/30/18 Lobar, no culture	10/29/18 Hemolytic strep.	Closure 3/24/19
13	10/ 2/18	Nasopharyngitis, acute	10/ 4/18 Lobar, hemolytic strep.	10/11/18 Hemolytic strep.	Closure 3/28/19
14	10/ 6/18	Nasopharyngitis, acute	10/ 7/18 Lobar, no culture	10/25/18 Hemolytic strep.	Closure 3/18/19
15	10/16/18	Lobar pneumonia	10/16/18 Lobar, pneumo. IV	10/24/18 Hemolytic strep.	Free drainage
16	10/18/18	Measles	10/29/18 Broncho., hemolytic strep.	11/ 5/18 Hemolytic strep.	Free drainage
17	10/23/18	Measles	11/ 1/18 Broncho., hemolytic strep.	11/ 4/18 Hemolytic strep.	Discharged 3/14/19
18	12/ 6/18	Tonsillitis	Jan. 1918 at Camp Meade, empyema 3/18/18	12/10/18 Recurrent emp., hem. strep.	Discharged 3/3/19
19	1/ 3/19	Bronchopneumonia	1/ 3/19 Broncho., streptococcus	1/15/19 Hemolytic strep.	Free drainage
20	1/14/19	Nasopharyngitis, tonsillitis	1/22/19 Broncho, pneumo. IV	2/ 6/19 Hemolytic strep.	Free drainage
21	1/15/19	Lobar pneumonia	1/15/19 Lobar, B. influenzae	1/23/19 Hemolytic strep.	Free drainage
22	1/18/19	Convalescent sup. pleurisy	Overseas history of influenzal pneumonia	10/28/18 Thoracotomy, Brest, France	Closure 3/25/19
23	1/27/19	Nasopharyngitis	1/28/19 Lobar, hemolytic strep.	2/ 2/19 Hemolytic strep.	Free drainage
24	1/28/19	Convalescent sup. pleuritis	Mumps, influenza, pneumonia, empyema October, 1918	11/11/18 Thoracotomy, Camp Merritt	Free drainage
25	2/ 6/19	Convalescent sup. pleuritis	10/18/18 Lobar (overseas)	11/19/18 Thoracotomy, Liverpool, Eng.	Free drainage

by a suppurative pleuritis. In all cases giving a history of pneumonia, the date on which the condition developed is accompanied by a statement of the diagnosed clinical type, together with the laboratory findings. The date of onset of the empyema, with the infecting organism, is followed by a statement of the status of the case, three months after the start of the investigation.

BLOOD PICTURE IN CHRONIC EMPYEMA

Before undertaking the bacteriologic study of these cases, it was deemed advisable to ascertain if this continued chronic condition had caused noticeable changes in the blood picture.

The blood in acute cases, which have been studied, shows invariably a leukocytosis varying from 15,000 to as high as 35,000 cells per c.mm. The average is about 20,000. In the acute condition, the differential count shows a neutrophilia of from 75% to 85%, small mononuclears representing 5% to 10% of white cells, and large mononuclears and transitionals about 10%. The presence of eosinophils is slightly above normal, with basophils present in normal proportion.

In the chronic cases, a leukocytosis was observed with counts ranging from 7,500-30,000, the average of all cases being 14,000 per c.mm., with 10,000 or thereabouts the usual count. The average for neutrophils was from 60% to 70%, with small mononuclears present in the proportion of 20% to 25%, and large mononuclears 10%. Eosinophils are found more commonly than in the acute cases, while basophils are rarely observed.

Total erythrocyte counts in acute cases could not be distinguished from a series of controls on normal individuals. In the chronic cases here reported, a secondary anemia was found to develop consistently, with counts below normal in almost every instance. Four cases showed counts of less than 4,000,000, the lowest being 3,200,000 per c.mm. The usual count was within the range of from 4,000,000-5,000,000. Hemoglobin estimation gave an average reading of 85%.

The presence of this secondary anemia may be attributed to the universal presence of an initial hemolytic streptococcus infection.

BACTERIAL CONTROL OF TREATMENT

In the treatment of suppurative pleuritis by irrigation with antiseptics, the variation of the treatment is directed to a large extent by the results attained in freeing the cavity of bacterial contamination. Routine weekly smears have been made during the course of treatment. The method employed is essentially that recommended in the Carrel-Dakin treatment of open wounds, but its application to empyema cavities presents some technical difficulties. At first smears were obtained by passing a platinum looped needle through the drainage tube, but this was soon discarded. It was realized that erroneous counts might well be obtained through growth of organisms within the rubber tube, and the collection of old pus, in cases where tubes were not removed and cleaned each day. Consequently the practice of first removing the tubes was adopted, and the needle was passed in through the external sinus. This likewise proved not altogether satisfactory, as it is difficult to prevent contamination of the needle around the opening and along the walls of the sinus, in entering the cavity. The making of the smear from the end of the drainage tube resting in the cavity was also tried, the smear being made immediately following its removal. Finally, a method suggested by the use of the West tube in meningococcus work, was developed. A long series of smears have been made simultaneously by the various methods outlined, and the bacterial counts compared. The following gives the most consistently satisfactory results:

The drainage tube is removed and is replaced by a sterile rubber tube much smaller in diameter than the tube removed. It is also shorter and should be about 5 cm. long, or just long enough to reach through the external sinus. A long looped needle is then introduced through this sterile tube into the cavity, the pus pocket is smeared, and the needle with its loop of pus, carefully withdrawn through the sterile tube. With this technic one eliminates all error due to old pus collected within the drainage tube, or along the walls of the sinus. A representative specimen of the fluid inside the pleural cavity is obtained. Before determining finally the bacteriologic fitness of the wound for closure, smears must be made from the sinus as well as the cavity.

Smears of the fluid were made on glass slides, stained with carbol-thionin, and examined. Large numbers of leukocytes are invariably present. They show signs of necrosis as a rule, and appear in various stages of degeneration. In smears from chronic cases there is rarely evidence of phagocytosis.

Counts of the bacteria present are made under the microscope, using the immersion lens.

The value of the bacteriologic control rests on a standardization of technic. A standard sized looped needle should be used, and the system of lenses, and tube length of the microscope must always be the same. This is necessary to obtain true comparative results, and a comparison of results is the basis on which this method rests, a comparative increase or decrease of bacteria in a standard field. It is believed that the method just outlined for obtaining the material for smears tends more nearly to standardize that part of the procedure. The rest of the technic is a matter of mechanical detail, leaving the personal equation in the actual counting as the variable factor.

TECHNIC OF CULTURE

Material for culture was obtained from the pleural cavity in the manner outlined for obtaining exudate for bacterial counts. Streak inoculations were made on plates in the manner recommended for meningococcus work.[11] The medium used was a beef infusion agar titrated to a P_H of 7.4-7.6, to which was added aseptically 5% of sterile horse blood. Direct smears were made at the time of culture, and the microscopic examination checked by the cultural results.

In all cases in which thoracotomy or rib resection was done from time to time, previous to operation 10 c c of fluid was withdrawn aseptically from the pus pocket with a sterile syringe. The fluid thus obtained was examined culturally and microscopically in the same way as exudate obtained from the open cavity.

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[11] Standard Methods for the Detection of Meningococcus Carriers, U. S. Army, 1918.

BACTERIOLOGY OF PLEURAL EXUDATES

A systematic program of culturing every case at weekly intervals was instituted, and has been followed through a period of 3 months.

Only one case of the series (11) gave a history of the pneumococcus as the etiologic factor. At the time of the first culturing in the present study, no cultural evidence of the pneumococcus could be found in that case, nor was it subsequently obtained in culture. In fact the pneumococcus has not been obtained in culture in any of the present work.

In the remaining 24 cases, hemolytic streptococci were the primary invaders causing the suppurative condition of the pleural cavity. The examination of the fluid at the time of the first aspiration demonstrated streptococci as the primary agents in the case of all those patients who were admitted from this command. In those cases transferred from overseas, or from other hospitals, the organism still persisted in the cavity at the time of admittance. However, as brought out later, with the persistence of the condition, it appears that the original flora is displaced in many instances by secondary invaders. At the time this investigation was undertaken, the hemolytic streptococcus could be demonstrated in only 11 cases of 24 of known streptococcal etiology.

Green-producing streptococci have been cultivated in something over half of the cases during the series of cultures taken, but have been found with no regularity, and in but one case do they seem to be the predominating organism. This type of streptococcus is among the most common secondary invaders. Their presence, in the small numbers in which they have been found, is probably of little significance.

By far the most interesting result is the finding in a large percentage of these old chronic cases of some member of a broad proteolytic group of bacilli. Nineteen of 25 show the presence of these organisms in culture and in smear.

Staphylococci in varying numbers have been found in practically all of the cases, and the majority of the cultures as they are taken from week to week will show a few colonies of the aureus or albus varieties.

Diphtheroids, usually of the solid staining, but occasionally of the barred type, are encountered from time to time, but never in large numbers, or predominating in a culture. Occasional colonies of chromogenic air bacteria have been found, as would be expected.

Such is the varied flora encountered.

The most significant results have been obtained in cultures derived from pus of secondarily encysted areas at the time of operation. Frequent fluoroscopy has denoted from time to time walled off areas of infection which had developed subsequent to the primary operation, often some months following it. A second thoracotomy was made to allow drainage of these pockets. In 10 of 11 fluids obtained at the time of operation, a proteolytic bacillus was found to be the predominating organism. Oftentimes it was obtained in pure culture, sometimes with an accompanying staphylococcus, and in two instances with a Streptococcus viridans as well as staphylococci. In all specimens, however, it was the predominating organism. Neither hemolytic streptococci nor pneumococci have been isolated from any of these fluids.

SEROLOGIC STUDY OF PATIENT'S BLOOD

The interesting results which were attained in the bacteriologic study of these empyemic lesions suggested that a more definite idea of the history and course of the infection might be deduced from a study of the blood.

A representative strain of hemolytic streptococcus was selected from a number isolated from cultures of chest fluids. It corresponded to Streptococcus pyogenes of the Holman classification. Macroscopic agglutination tests, using this strain, were made with the serum of patients. The results were most interesting. Twenty serums were tested, and all showed positive agglutination for this streptococcus. Four serums showed agglutination at a dilution of 1:400, in 10 cases the titer proved to be 1:800, and in 5 cases, 1:1,600. For one serum a titer of 1:3,200 was demonstrated. All of these serums were from cases of initial streptococcic empyema. At the time of making the tests, streptococci were absent from cavity culture in all but one case. The general absence in culture, and the uniform high titer for the organism, would indicate that the body had established a well fixed immunity for that organism.

As has been noted, streptococci of the green producing type have been obtained in culture in numerous instances, and in a number of the cases. Agglutination tests, employing a like technic, were made with the same serums, using this time as the agglutinogen, a strain of Streptococcus viridans isolated in the course of the work. No antibodies, even in a dilution of 1:10, could be demonstrated in any serum.

A slight antibody content for one or the other of the two types of proteolytic bacilli to be described later, was found present in seven serums, all from cases in which the organism had been demonstrated in culture from the cavity. In only one case did agglutination occur in a higher serum dilution than 1 : 20. A positive reaction at a dilution of 1 : 40 was obtained in that instance.

As controls on these four sets of agglutinations, like tests were made with serum from 15 normal individuals. No agglutinins for the same strain of streptococcus employed in the first experiment were demonstrable. Neither were agglutinins for Streptococcus viridans or either group of proteolytic bacilli present.

The relative frequency of tuberculous complications in protracted empyemas has been emphasized. To determine the possibility of tuberculous empyema being the cause of some of these chronic cases, complement fixation tests for the tubercle bacillus were made with negative results in all cases. Sputum examinations likewise failed to demonstrate the presence of B. tuberculosis.

Routine Wassermann tests also proved negative in every instance.

HEMOLYTIC STREPTOCOCCI IN CHRONIC EMPYEMA

The importance of hemolytic streptococcus in primary pleural infections has been thoroughly demonstrated by the reports of its presence in the majority of cases throughout the country. The cases here reported have all shown it in the first culture with one exception. The importance of the streptococcus in the development of the chronic state is open to question. It will be remembered that only 11 of the 25 cases here reported have at any time during this investigation given positive cultures of the organism. Of these 11 cases which gave positive findings at the beginning of the study, only 1 (22) continued to show the organism 3 months later. Eight cases (2, 6, 10, 16, 19, 20, 23 and 25) continued free drainage at that time, with discharge of fetid pus, although hemolytic streptococci had not been demonstrated in culture for a minimum of 4 weeks, and in some instances longer. Two patients (3 and 12) have been discharged to duty as recovered, streptococci having been present in only the early cultures. In but 1 case can the persisting condition be attributed to the streptococcus.

In this entire study the persistence of hemolytic streptococci has been of shorter duration, under daily irrigations of antiseptic solution, than that of the group of proteolytic bacilli already mentioned. Taken in general, the flora of these wounds seemed to follow, under treatment with antiseptic irrigations, a course much as follows: The first cultures showed hemolytic streptococci in pure culture. Under treatment following operation this organism was gradually replaced by a varying flora, usually staphylococci first being found with the streptococci, and occasionally a few diphtheroids. Soon some member of the proteolytic group became the predominating organism in culture. It continued so under treatment until gradually eliminated by the action of the antiseptic. With the disappearance of the later organisms, the discharge decreased in amount. The end result was a small amount of secretion, viscid and translucent, and serous in nature. Culture showed only an occasional staphylococcus colony, with a very low bacterial smear count. Closure of the wound followed.

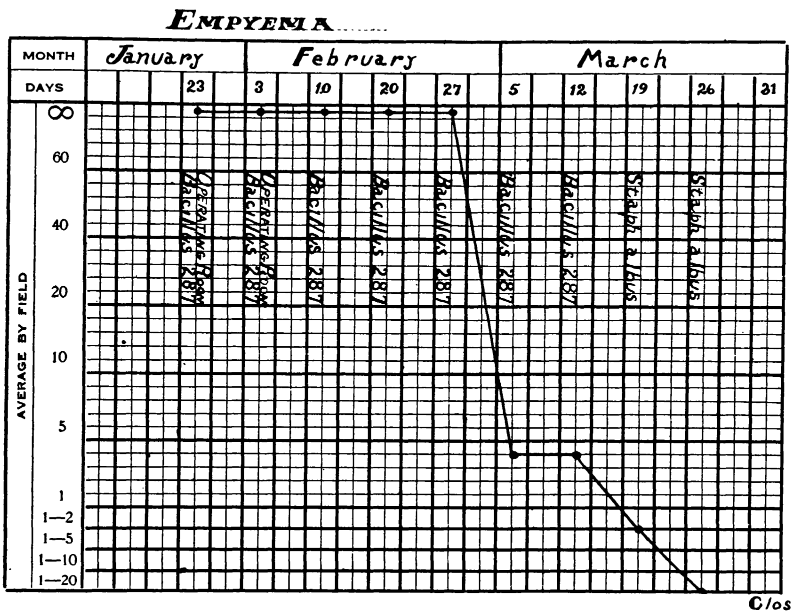

Fig. 1.—Typical bacterial count and flora in chronic empyema.

These results are at variance with the findings of the empyema commission working at Camp Lee. The members of the commission state that hemolytic streptococci are by no means the first organisms to disappear under antiseptic treatment in mixed infection of cavities neglected after operation, and likewise that putrefactive organisms and B. pyocyaneus disappear much earlier than hemolytic streptococci, as indicated by plate cultures. The results which I have attained from weekly cultures of wounds, and from the culture of secondarily walled off pockets, at the time of operation, would seem to ascribe a greater importance to the heterologous group of proteolytic bacilli, especially a predominating organism of the Friedländer group, than to streptococci as the bacterial factor in protracted empyema.

No detailed study of the streptococci isolated from these cases has been undertaken, but in connection with some other work on streptococci, sugar reactions were determined on strains picked from plate cultures, all of the hemolytic type.

Inoculations were made on a beef infusion broth to which 5% of horse serum and 1% of Andrade indicator had been added. Twenty per cent. solutions of the sugars were sterilized at 100 C. for three successive days, and added in sufficient amount to give a final 1% sugar medium. Holman's classification was employed in grouping the strains. Nine of 13 tested were found to be Streptococcus pyogenes, 2, S. hemolyticus III, and 3, S. infrequens.

Serum against strains of S. hemolyticus III, and against S. pyogenes, were used in agglutination tests. No differential characteristics could be observed. Positive agglutinations were obtained in a serum dilution of 1:2,500 irrespective of heterogeneous or homogeneous serum. The results bear out the accepted conclusions that agglutination reactions are of little value in the differentiation of streptococci.

PUTREFACTIVE BACTERIA

The consistent finding in successive cavity cultures, from 19 of 25 cases, and the universal observation in operative fluids, of these proteolytic bacilli, seemed to warrant a closer and more exact stüdy of their biologic nature.

BACILLUS PROTEUS

In 5 of these cases I have isolated a proteus bacillus. It was also obtained late in the course of 2 other cases in which the organism to be described later predominated. It agrees culturally with the usual B. proteus vulgaris, fermenting glucose and saccharose with gas production, but showing no action on lactose. Morphologically, it is a typical proteus bacillus, and shows motility. It seems to be a variant of the common type of B. proteus, for it is wholly inagglutinable by an immune serum produced with a known strain as antigen. Furthermore, it shows a well developed tendency to retain the Gram stain.

Still, agglutination reactions among various strains of the proteus group are always variable, and lack of agglutinative power was rather to be expected. All of the strains isolated from empyema are, however, agglutinated in at least a dilution of 1 : 1,000 by an immune serum produced by one of the strains (192). Results indicate that these 7 strains encountered in the course of the work represent a single type of the large and varying proteus group.

BACILLUS OF FRIEDLÄNDER GROUP

A small gram-negative bacillus has been obtained in 14 cases by wound culture, and in 10 cases from fluids drawn from cavities just prior to operation.

Morphology.—The bacillus is a rather broad, rod-shaped organism, the length not exceeding 1-2 mikrons. Sometimes, in old cultures especially, besides the coccobacillary forms, longer and even filamentous bacilli are observed. The organism is nonmotile and spores are never found. Capsule formation is irregularly observed, but can usually be demonstrated in growths from animal fluids, rarely from artificial culture. The bacillus stains readily with all of the basic anilin dyes, and is negative to Gram's stain.

Cultural Characteristics.—The organism is a facultative anaerobe, and grows very luxuriantly on all culture mediums. Blood agar was used in all primary cultures, but equally good growths are obtained on beef extract agar, and on plain broth. Colonies on agar plates develop in a few hours. The growth appears in the form of grayish white, mucous-like colonies, having a characteristic slimy and semifluid appearance. Colonies have a marked tendency to become confluent, so that the surface of plates even at the end of 24 hours has the appearance of being covered with a glistening sticky exudate. The odor given off by cultures is decidedly penetrating and disagreeable, and of that distinctive quality characteristic of the Friedländer group. Cultures on agar remain viable over long periods. Gelatin is liquefied, as is Loeffler's blood serum. Evidence of hemolytic action on blood medium is present, but may be due to the marked proteolytic enzymic action, rather than to any distinct hemolytic power. Milk is an excellent medium for the growth of the bacillus. It is rendered alkaline with no coagulation. Proteolysis at the end of 5 days is practically complete, and partial reduction of the litmus is present.

Fermentation Reactions.—The various strains isolated were found by sugar reactions, and later by serologic tests, to be differentiated into one main group, and a smaller sub-group or variant. Strains from 12 cases fermented glucose without gas production. Lactose was not affected. Saccharose was fermented but without the production of gas. Acid production in inulin was always present. Strain 287, isolated from a secondary pus pocket at operation, is representative of the organism described.

Strains consistently present in 2 cases differed from the usual organism found in that gas as well as acid production followed in glucose and saccharose mediums, with lactose remaining unaffected as usual. Inulin was unaffected by these strains. Like strains were isolated in 4 other instances from cases in which the usual nongas-producing bacillus was consistently present. Culturally, the organisms were otherwise identical. Strain 086 of table 2, likewise isolated at operation, is typical of this variant.

Serologic Reactions.—An immune rabbit serum was developed against strain 287. The serum gave a titer of 1:6,000 against the homologous organism. All strains isolated were tested by agglutination against this serum, and in each instance reacted positively. All strains culturally the same, in that sugar reactions showed absence of gas production, were agglutinated by the serum in at least a dilution of 1:1,000. Those strains producing acid and gas in glucose and saccharose, and seemingly denoted as being a variant of the bacillus, were found to show agglutination at 1:20 and 1:40 dilution only. Aserum developed against strain 086, a gas producing type, agglutinated all of that variety in a serum dilution of at least 1:1,000, with low cross agglutination for the nongas-producing type.

The sugar and serologic reactions of these gas-producing strains are sufficient to distinguish them from the nonfermenting type. The morphology and growth characteristics are otherwise identical. It would seem that this smaller group was a variant of the main group. That it is closely allied is denoted by its cross agglutination, serum 287 agglutinating strain 086, and vice versa, serum 086 showing cross agglutination with strain 287. Cross agglutination with B. proteus vulgaris, B. mucosus capsulatus, and B. rhinoscleromatis was entirely absent.

Virulence.—White mice are killed by 0.001 c c of a 24-hour old broth culture of the bacillus, when injected intraperitoneally. One cubic centimeter of a like culture proves fatal for guinea-pigs of about 300 gm. weight. It is less virulent for rabbits.

Distribution.—In addition to being commonly found in these cases of chronic empyema, the organism has also been observed in 2 cases of pneumonia, typed pneumococcus I, and in a case of influenza; all encountered in the course of routine cultural work.

TABLE 2

CULTURAL CHARACTERISTICS

Organism	Glucose		Lactose		Saccharose		Maltose		Galactose		Mannite		Inulin	Litmus Milk	Gelatin
	Gas.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid			
B. proteus vulgaris.....	+	+	—	—	+	+	+	+	+	+	—	—	—	Acid Coagulation Proteolysis	+
B. mucosus....	+	+	—	—	+	+	+	+	+	+	—	—	—	Acid Coagulation Proteolysis	—
B. rhinoscleromatis......	—	+	—	—	—	+	—	+	—	+	—	—	—	Alkaline No coagulation No proteolysis	—
B. proteus 192	+	+	—	—	+	+	+	+	+	+	—	—	—	Acid Coagulation Proteolysis	+
Bacillus 086....	+	+	—	—	+	+	+	+	+	+	—	—	—	Alkaline Reduction litmus Proteolysis	+
Bacillus 287....	—	+	—	—	—	+	—	+	—	+	—	—	+	Alkaline Reduction litmus Proteolysis	+

Table 2 shows the cultural reactions of this organism, its variant 086, as well as those of the proteus bacillus isolated in this study. Comparison is afforded with the cultural characteristics of known strains of B. proteus vulgaris, B. mucosus capsulatus of Friedländer, and B. rhinoscleromatis.

The exact classification of this organism is undetermined. The main group is clearly differentiated from the proteus group by its lack of gas production in any of the sugar mediums. It is related to that general group of bacilli of which B. mucosus is the dominating member. The close relationship of B. mucosus, B. rhinoscleromatis and the ozena bacillus serves to place them in a general group, the Friedländer or capsulated group. As indicated in the table, this bacillus resembles most closely B. rhinoscleromatis and the ozena bacillus, but differs from either by its rapid liquefaction of gelatin. The gas producing type is a variant in the direction of B. mucosus capsulatus itself. Biologically, the bacillus would seem to fall into the general Friedländer group. Its biochemical and cultural reactions, however, serve to differentiate it as a distinct species.

SUMMARY AND CONCLUSIONS

The blood picture in chronic empyema, in contradistinction to that of acute cases where the leukocytosis is high with a high percentage of polymorphonuclears, gives a low grade leukocytosis of about 10,000 white cells per c.mm. and a neutrophilia of 60-70%. A secondary anemia is invariably present in old and protracted cases.

A new technic for obtaining smears for bacterial counts from the pleural cavity is described. The use of a sterile protective tube in entering the cavity through the external sinus gives more uniform and consistent results in bacterial counts for the control of treatment.

Hemolytic streptococci are the usual causative agent in the series of cases here reported. These findings are in accordance with general observations in military practice of the past two years.

Study of the cases at Camp Gordon would seem to indicate that hemolytic streptococci play a minor part in empyemas which have developed into the chronic state. Fetid, purulent discharges continue to be given off, and closure of the wound is impossible, long after the hemolytic streptococcus is absent in culture or smear from wound or secondary operative fluid. A high degree of immunity to hemolytic streptococci has been demonstrated in these cases.

A heterologous group of proteolytic bacilli, of which one group predominates, has been observed in a large percentage of cases.

The typical bacterial picture in the present cases seems to be (1) hemolytic streptococci in pure culture, (2) gradual supplanting of the streptococci by a flora of secondary invaders, and (3) freeing of the wound of these organisms under treatment, followed by closure.

A variety of the proteus group has been isolated from this secondary flora of the cavity, and group classification established.

The predominating organism among secondary invaders has in these cases been a gram-negative bacillus, broadly belonging to the Friedländer group. The reactions and cultural characteristics have been described.